



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: A61K 39/12	A1	(11) International Publication Number: WO 98/10790 (43) International Publication Date: 19 March 1998 (19.03.98)
(21) International Application Number: PCT/US97/15820 (22) International Filing Date: 8 September 1997 (08.09.97) (30) Priority Data: 60/025,221 11 September 1996 (11.09.96) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): GUPTA, Sunil, K. [IN/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). ALVES, Kenneth [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MARK, George, E., III [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). PATEL, Mayur, D. [GB/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PAPILLOMAVIRUS VACCINE FORMULATION (57) Abstract An immunogenic composition comprising papillomavirus virus-like particles comprised of recombinant papillomavirus L proteins mixed with recombinant papillomavirus early proteins is provided.		

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TITLE OF THE INVENTION

PAPILLOMAVIRUS VACCINE FORMULATION

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 shows the effects of L1, L2 and E protein immunization on CRPV induced papilloma regression.

 Figure 2 shows antibody titers in rabbits immunized with VLPs and E-proteins.

10 Figure 3 shows the effects of L1/L2 VLPs and E proteins on papilloma development when animals were challenged with undiluted virus stock.

 Figure 4 shows the effects of L1/L2 VLPs and E proteins on papilloma development when animals were challenged with virus stock diluted 1:4.

15

BACKGROUND OF THE INVENTION

 The papillomaviruses (PV) are a group of viruses that produce papillomas or warts in a variety of higher vertebrates, including humans, cattle, rabbits, horses, dogs, and nonhuman primates.
20 In addition, papillomaviruses have been linked to a variety of naturally-occurring cancers in various animal species, including humans.

 Papillomaviruses are small, nonenveloped, double-stranded DNA viruses with icosahedral symmetry that replicate solely in the nucleus of infected cells. The papillomaviruses (PVs) are remarkably
25 tissue- and species-specific (Howley, 1990). Most can replicate only in squamous epithelial cells, and there are no described instances of a papillomavirus from one species causing a productive infection in a different species (Howley, 1990). Thus, humans are the only natural hosts for human papillomaviruses (HPVs).

30 More than 75 different types of HPVs have been identified. Different HPV types are characteristically associated with specific lesions. Thus, distinct HPV types are responsible for common skin warts, plantar warts, respiratory papillomas of juvenile onset, squamous cell carcinomas arising in patients with epidermodysplasia

verruciformis (a rare dermatological disorder), and genital warts. Among the HPVs that infect the lower genital tract, HPV-6 and HPV-11 are frequently associated with subclinical infections and condylomata acuminata or benign genital warts; HPV 6 and HPV 11 are rarely seen
5 in higher-grade neoplasias and cervical carcinomas (Shah and Howley, 1990). HPV-16 and HPV-18, on the other hand, predominate in high-grade anogenital lesions and invasive cancers of the cervix, vulva, penis, and anus (Shah and Howley, 1990).

Human papillomavirus infection of the genital tract is one
10 of the most common sexually transmitted diseases. HPV infection of the genital tract may be easily recognized by the appearance of genital warts.

Several lines of evidence highlight the importance of the immune system's response, particularly cell-mediated immunity, in the
15 course of papillomavirus infections (Shah and Howley, 1990). Individuals having depressed T-cell function (e.g., AIDS patients, pregnant women, and patients undergoing immunosuppressive therapy or organ transplantation) are reported to have a higher prevalence of warts as well as an increase in the size and number of warts. In
20 addition, warts often regress completely when the immunosuppressed state is reduced or eliminated. Finally, histological sections of regressing flat skin warts show a mononuclear cell infiltrate suggestive of an immune-mediated response.

Effective prophylactic and therapeutic vaccines containing
25 the L1 and L2 proteins of bovine papillomavirus (BPV) 2 have been formulated (Jarrett et al., 1991). Cottontail rabbits have been immunized with cottontail rabbit papillomavirus (CRPV) virus-like particles (VLPs; particles that are morphologically similar to native virions but that lack viral DNA). VLPs are formed from recombinant
30 L1 protein (L1 protein is the major component of the PV viral capsid). Immunization of rabbits with CRPV VLPs (Christensen et al., 1996) or with CRPV L1 or L2 proteins (Lin et al., 1992) elicits neutralizing antibodies that protect rabbits from papilloma formation following

challenge with native CRPVs. The neutralizing antibodies do not, however, prevent disease progression in preexisting CRPV infection.

HPV infection in nonhuman animals does not produce disease. This necessitates the use of animal PV models for the testing of candidate vaccines. The cottontail rabbit-CRPV model is a well-characterized in vivo PV system; it displays a remarkable similarity to the natural history of HPV-induced diseases (Höpfl et al., 1995). Both CRPV and HPV infections have a high incidence of spontaneous regression (10-40%) and of malignant progression of persistent papillomas (Höpfl et al., 1995). This similarity makes the CRPV model particularly well suited for the development of a human anticancer vaccine.

One objective of the instant application is the development of an HPV VLP vaccine that is both prophylactic--that is, prevents HPV infection--and therapeutic--provides immunotherapy to patients with preexisting HPV infection. In order to prevent HPV infection, the vaccine must elicit neutralizing antibodies that preclude viral entry into cells. As the CRPV model indicates, such antibodies are produced in response to injection with CRPV VLPs. For the vaccine to be therapeutic, it must elicit a cell-mediated immune response targeted toward the specific HPV antigens; such a response will kill HPV-infected cells, thereby preventing disease progression. The induction of cell-mediated immunity is accomplished via the injection of viral E proteins, which must be taken up by the cell, processed, and presented complexed with the appropriate MHC Class I molecules by antigen-presenting cells. Thus, the prophylactic/therapeutic HPV vaccine will consist of VLPs in combination with the appropriate HPV E proteins. In order to test this formulation we generated CRPV L1/L2 VLPs expressed in baculovirus or yeast and E1, E2, E5, E6, and E7 proteins expressed in Escherichia coli for testing in the in vivo CRPV model.

SUMMARY OF THE INVENTION

An immunogenic composition comprising papillomavirus virus-like particles comprised of recombinant papillomavirus L proteins mixed with recombinant papillomavirus early proteins is provided.

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DETAILED DESCRIPTION OF THE INVENTION

An immunogenic composition comprising papillomavirus virus-like particles comprised of recombinant papillomavirus L proteins mixed with recombinant papillomavirus early proteins is provided.

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Papillomavirus infections occur in a variety of animals, including humans, sheep, dogs, cats, rabbits, monkeys, snakes and cows. Papillomaviruses infect epithelial cells, generally inducing benign epithelial or fibroepithelial tumors at the site of infection.

Papillomaviruses may be classified into distinct groups based on the host that they infect. Human papillomaviruses (HPV) are further classified into more than 60 types based on DNA sequence homology (for a review, see Papillomaviruses and Human Cancer, H. Pfister (ed.), CRC Press, Inc., 1990). Papillomavirus types appear to be type-specific immunogens in that a neutralizing immunity to infection to one type of papillomavirus does not confer immunity against another type of papillomavirus.

In humans, different HPV types cause distinct diseases. HPV types 1, 2, 3, 4, 7, 10 and 26-29 cause benign warts in both normal and immunocompromised individuals. HPV types 5, 8, 9, 12, 14, 15, 17, 19-25, 36 and 46-50 cause flat lesions in immunocompromised individuals. HPV types 6, 11, 34, 39, 41-44 and 51-55 cause nonmalignant condylomata of the genital and respiratory mucosa. HPV types 16 and 18 cause epithelial dysplasia of the genital tract and are associated with the majority of *in situ* and invasive carcinomas of the cervix, vagina, vulva and anal canal. HPV6 and HPV11 cause the majority of genital warts and laryngeal papillomas.

30

Immunological studies in animals have shown that the production of neutralizing antibodies to papillomavirus capsid proteins prevents infection with the homologous virus. The development of

effective papillomavirus vaccines has been slowed by difficulties associated with the cultivation of papillomaviruses in vitro. The development of an effective HPV vaccine has been particularly slowed by the absence of a suitable animal model.

5 Neutralization of papillomavirus by antibodies appears to be type-specific and dependent upon conformational epitopes on the surface of the virus.

Papillomaviruses are small (50-60 nm), nonenveloped, icosahedral DNA viruses that encode for up to eight early and two late
10 genes. The open reading frames (ORFs) of the virus genomes are designated E1 to E7 and L1 and L2, where "E" denotes early and "L" denotes late. L1 and L2 code for virus capsid proteins. The early (E) genes are associated with functions such as viral replication and transformation.

15 The L1 protein is the major capsid protein and has a molecular weight of 55-60 kDa. L2 protein is a minor capsid protein which has a predicted molecular weight of 55-60 kDa and an apparent molecular weight of 75-100 kDa as determined by polyacrylamide gel electrophoresis.

20 The present invention is directed to the vaccines and immunogenic compositions comprising mixtures of recombinant papillomavirus proteins having the immunity conferring properties of native papillomaviruses. The present invention is particularly directed to prophylactic and therapeutic vaccine formulations. The present
25 invention is exemplified by a cottontail rabbit papillomavirus (CRPV) model system. The exemplification does not limit the scope of the invention, which includes other types and subtypes of papillomavirus (PV), including but not limited to HPV type 11, HPV type 16 and HPV type 18 as well as HPV subtype 6a and HPV subtype 6b.

30 Pharmaceutically useful compositions comprising the proteins or VLP may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically

acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein or VLP. Such compositions may contain proteins or VLP derived from more than one type of HPV.

5 Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose PV infections. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. Generally, the compositions
10 will be administered in dosages ranging from about 1 μ g to about 250 μ g.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral, mucosal, and intramuscular.

15 The vaccines of the invention comprise recombinant proteins or VLP that contain the antigenic determinants necessary to induce the formation of neutralizing antibodies in the host. Such vaccines are also safe enough to be administered without danger of clinical infection; do not have toxic side effects; can be administered by
20 an effective route; are stable; and are compatible with vaccine carriers.

The vaccines may be administered by a variety of routes, such as orally, parenterally, subcutaneously, mucosally or intramuscularly. The dosage administered may vary with the condition, sex, weight, and age of the individual; the route of administration; and
25 the type PV of the vaccine. The vaccine may be used in dosage forms such as capsules, suspensions, elixirs, or liquid solutions. The vaccine may be formulated with an immunologically acceptable carrier.

The vaccines are administered in therapeutically effective amounts, that is, in amounts sufficient to generate a immunologically
30 protective response. The therapeutically effective amount may vary according to the type of PV. The vaccine may be administered in single or multiple doses.

The methods of the present invention make possible the formulation of subviral vaccines for preventing or treating PV infection.

5 The recombinant proteins and VLP of the present invention may be used in the formulation of immunogenic compositions. Such compositions, when introduced into a suitable host, are capable of inducing an immunologic response in the host.

10 The recombinant proteins and VLP may be used to generate antibodies. The term "antibody" as used herein includes both polyclonal and monoclonal antibodies, as well as fragments thereof, such as, Fv, Fab and F(ab)₂ fragments that are capable of binding antigen or hapten.

15 The following examples are provided to further define the invention without, however, limiting the invention to the particulars of these examples.

EXAMPLE 1

Subcloning and expression of CRPV E1, E2, E4, E5, E6 and E7 genes in *E. coli*

20 PCR primers based on the published sequence of CRPV (Yaniv, M., Danos, O. and Giri, I., "Genomic Structure of the Cottontail Rabbit (Shope) Papillomavirus", Proc. Natl. Acad. Sci. U.S.A., 82, 1580-1584 (1985)) were used to PCR amplify the full length CRPV E1, E2, E4, E5, E6 and E7 genes. To enhance expression
25 of CRPV E4 protein, the first 4 amino acid codons of CRPV E1 protein were fused to the amino terminal portion of E4 using PCR (E1⁴). To co-express E6 and E7 the two genes were fused at the carboxy terminus of E6 with the amino terminus of E7 using PCR. All PCR amplified products were subcloned into the vector pQE30 (Qiagen, Inc., San
30 Diego, CA) and then transformed into *E. coli*.

To express these genes 1 Liter cultures of *E. coli* expressing desired E-proteins were grown for eight hours at 37°C in LB media and then induced overnight at 30°C using 1 mM IPTG. The cells were then collected by centrifugation for 15 min at 5000 rpm, washed with 500 ml

of PBS and the E proteins were purified using the manufacturer's directions (Qiagen, Inc.)

EXAMPLE 2

5 Subcloning and expression of CRPV L1 and L2 genes as virus like particles (VLPs)

PCR primers based on the published sequence (Yaniv, M., Danos, O. and Giri, I., "Genomic Structure of the Cottontail Rabbit (Shope) Papillomavirus", Proc. Natl. Acad. Sci. U.S.A., 82, 1580-1584
10 (1985)) of CRPV were used to PCR amplify the full length CRPV L1 gene and a CRPV L2 gene that had the first 37 codons (111 bp) deleted from the virus genome. These genes were subcloned into the 2 cassette vector pAcUW51 (PharMingen Inc., San Diego, CA) for co-expression in the baculovirus expression system or into a 2 cassette vector pGAL-
15 110 (K. J. Hofmann et al. J. Virol. 209:506-518, 1995) for co-expression in yeast.

The pAcUW51 vector containing the genes encoding CRPV L1 and L2 proteins was transfected into SF9 cells using the PharMingen Baculogold expression kit. Supernatants from this transfection were
20 used to infect large cultures (1 Liter) of Sf9 cells which grown for 5 days, the cells were collected and the L1/L2 VLPs were purified.

Purification of VLPs from Sf-9 cells:

Cells were collected by centrifugation at 3800 x g for 30
25 min and were resuspended in phosphate-buffer saline pH 7.2 (PBS) at a density of 5×10^7 cells/ml. 10% NP-40 was added to 2% final volume (v/v) and the cells were stirred for 20 min in cold room. The cell slurry was then centrifuged at 12,000 x g for 30 min; the nuclear pellet was then collected and resuspended in PBS as above. Nuclei were
30 sonicated for 30 sec., and then $MgCl_2$ was added (to 3 mM), Benzonase was added (to 100 units/ml) and Pefabloc was added (to 0.2 mM). The nuclei were incubated for 6 hrs at room temperature while being mixed and then centrifuged at 12,000 x g for 30 min. Supernatant was collected and CsCl was added to the supernatant to a final concentration

of 0.48g/ml, centrifuged in a SW41 rotor at 30,000 rpm for 50 hrs and the VLP band was collected and dialyzed against PBS to remove CsCl. VLPs were further purified by anion exchange chromatography.

5 Expression of L1/L2 VLPs in yeast:

 The pGAL-110 vector containing the genes encoding CRPV L1 and L2 proteins was transformed into yeast using the standard spheroplast transformation protocol. Positive clones were identified and large scale cultures were grown. 200 ml of culture media (6.7 g/L
10 Yeast Nitrogen Base without amino acids or ammonium sulfate, 0.3 g/L adenine, 0.2 g/L L-tyrosine, 0.16 g/L uracil, 7.9 g/L succinic acid, 0.1 g/L L-arginine, 0.05 g/L L-Histidine, 0.3 g/L L-isoleucine, 0.2 g/L L-lysine, 0.05 g/L L-methionine, 0.3 g/L L-phenylalanine, 0.2 g/L L-tryptophan, 4% glucose) were inoculated with L1/L2- expressing yeast
15 cultures (recombinant yeast transformed with L1/L2/pLS110 vector) and grown for 2 days at 30°C. This 200 ml culture was then used to inoculate 1 liter of induction medium (2% yeast extract, 1% soy peptone, 1.6% glucose, 4% galactose) which was grown at 30°C for 5 days. The cells were collected by centrifugation and the L1/L2 VLPs
20 were purified by the procedure described above.

EXAMPLE 3

Immunization of Rabbits with Candidate Vaccine

 Rabbits were immunized with 50 ug of each CRPV L1/L2,
25 L1/L2+50 ug of each E1 and E2 or 50 ug of each E1 and E2 in 1.0 ml (0.3 ml each intramuscularly into each hind leg, 0.05ml each intradermally at 6 sites and 0.1 ml subcutaneously in the neck) of RIBI triple mix (Monophosphoryl Lipid A, Synthetic Trehalose
Dicorynomycolate and Cell Wall Skeleton, RIBI ImmunoChem
30 Research, Inc. Hamilton, MT). Rabbits were infected with cottontail rabbit papillomavirus (CRPV) 4 days after the first immunization and boosted with the same amount of antigen in the same formulation on day 21 and day 70. Size of the papillomas was determined at two week intervals beginning six weeks after the virus infection.

EXAMPLE 4

We tested in cottontail rabbits the ability of vaccination with nonstructural cottontail rabbit papillomavirus (CRPV) proteins E1 and E2, alone or in combination with L1/L2 VLPs, in limiting CRPV-induced papilloma development. Rabbits were immunized, using RIBI adjuvant, with L1/L2 VLPs, E1+E2 proteins, or the combination of VLPs and E-proteins, then boosted 3 weeks later with a similar dose of antigens in the same formulation. Animals were challenged 4 days after the initial antigen injection with two different concentrations of the CRPV virus. Rabbits vaccinated with L1/L2 VLPs or E.coli expressed E-proteins responded with antibody titers to the respective antigens, and developed papillomas at the sites of both high and low dose virus challenge. The rates of papilloma development in these vaccinees were indistinguishable from those of the unimmunized animal controls. However, the RIBI-adjuvanted vaccination of animals with L1/L2 VLPs in combination with E.coli expressed E1 and E2 proteins resulted in the retardation of papilloma development. In 5 of 9 animals the papillomas were reduced in size by greater than 80%, when compared with those of the control animals. This outcome suggests that the immunization with VLPs and E-proteins induced potent immune responses to virus-infected cells. This vaccination protocol had minimal to no effect on the papilloma development on the remaining 4 immunized rabbits. We hypothesize that this result may be due to variations in their major histocompatibility complex (MHC) receptors. These studies are continuing, evaluating additional adjuvants and E proteins. Studies are ongoing which investigate the utility of alternative adjuvants and E-proteins. Experiments are underway in which rabbits have been immunized with a cocktail of E1+E2+E1⁴, E6⁷ and E5 proteins in combination with VLPs in RIBI. Immunization of rabbits with VLPs and E1 and E2 proteins formulated in 0.1% Polyphosphazine also inhibits CRPV induced wart development in 3 of 4 rabbits. However, the magnitude and duration of inhibition is less than one observed in animals immunized with the same dose in RIBI.

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising virus-like particles containing papillomavirus L1 protein, papillomavirus L2 protein and at least one papillomavirus early protein.
2. The composition of Claim 1 wherein the early protein is selected from papillomavirus E1, E1⁴, E2, E3, E4, E5, E6, and E7 proteins.
3. The composition of Claim 2 wherein the papillomavirus is selected from human papillomavirus and cottontail rabbit papillomavirus.
4. The composition of Claim 1 which is a vaccine.
5. The composition of Claim 2 which is a vaccine.
6. The composition of Claim 3 which is a vaccine.
7. A method of inducing an antipapillomavirus immune response in an animal comprising administration of the immunogenic composition of Claim 1 to the animal.
8. The composition of Claim 1 which further comprises RIBI triple mix adjuvant.
9. The composition of Claim 2 which further comprises RIBI triple mix adjuvant.

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Effect of L1, L2 and E protein immunization on CRPV induced papilloma regression			
Rabbit #	Antigen	Mean Papilloma Size	Mean Papilloma Size
		LxW (mm ²)	LxWxH (mm ³)
1	RIBI	442 SD 329	4575 SD 3935
2	RIBI	326 SD 101	2229 SD 1266
3	RIBI	490 SD 11	3900 SD 962
4	RIBI+L1/L2	233 SD 122	1679 SD 1427
5	RIBI+L1/L2	547 SD 50	4626 SD 133
6	RIBI+L1/L2	697 SD 57	8042 SD 1024
7	RIBI+L1/E5-L2	427 SD 151	2723 SD 1209
8	RIBI+L1/E5-L2	744 SD 166	7438 SD 1657
9	RIBI+L1/E5-L2		
10	RIBI+E1+E2	600 SD 93	6000 SD 926
11	RIBI+E1+E2	668 SD 136	7470 SD 2213
12	RIBI+L1/L2+E1+E2	72.25 SD 64	255 SD 252
13	RIBI+L1/L2+E1+E2	600 SD 107	7700 SD 748
14	RIBI+L1/E5-L2+E1+E2	512 SD 14	3187 SD 601
15	RIBI+L1/E5-L2+E1+E2	165 SD 96	848 SD 695

FIG. 1

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Antibody titers in rabbits immunized with VLPs and E-proteins				
Rabbit #	Antigen	Anti-L1 Titers (Day 4)	Anti-E1 Titers (Day 81)	Anti-E2 Titers (Day 81)
101	RIBI	<100	<1600	<400
106	RIBI	<100	<400	<200
102	RIBI+L1/L2	<100	<200	<400
109	RIBI+L1/L2	<100	<16,00	<200
103	RIBI+L1/L2 +E1+E2	<100	12,800	25,600
105	RIBI+L1/L2 +E1+E2	<100	—	12,800
110	RIBI+L1/L2 +E1+E2	<100	3,200	12,800
113	RIBI+L1/L2 +E1+E2	<100	12,800	<25,600
114	RIBI+L1/L2 +E1+E2	200	n.d.	n.d.

FIG.2

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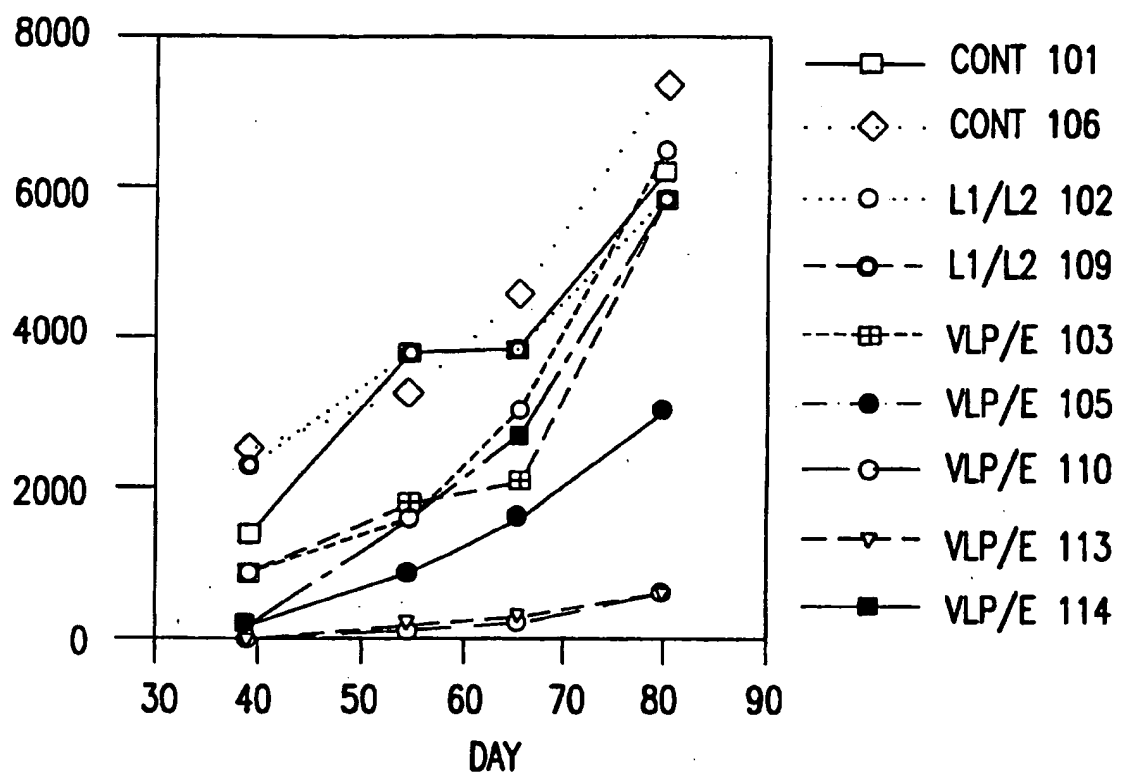


FIG.3

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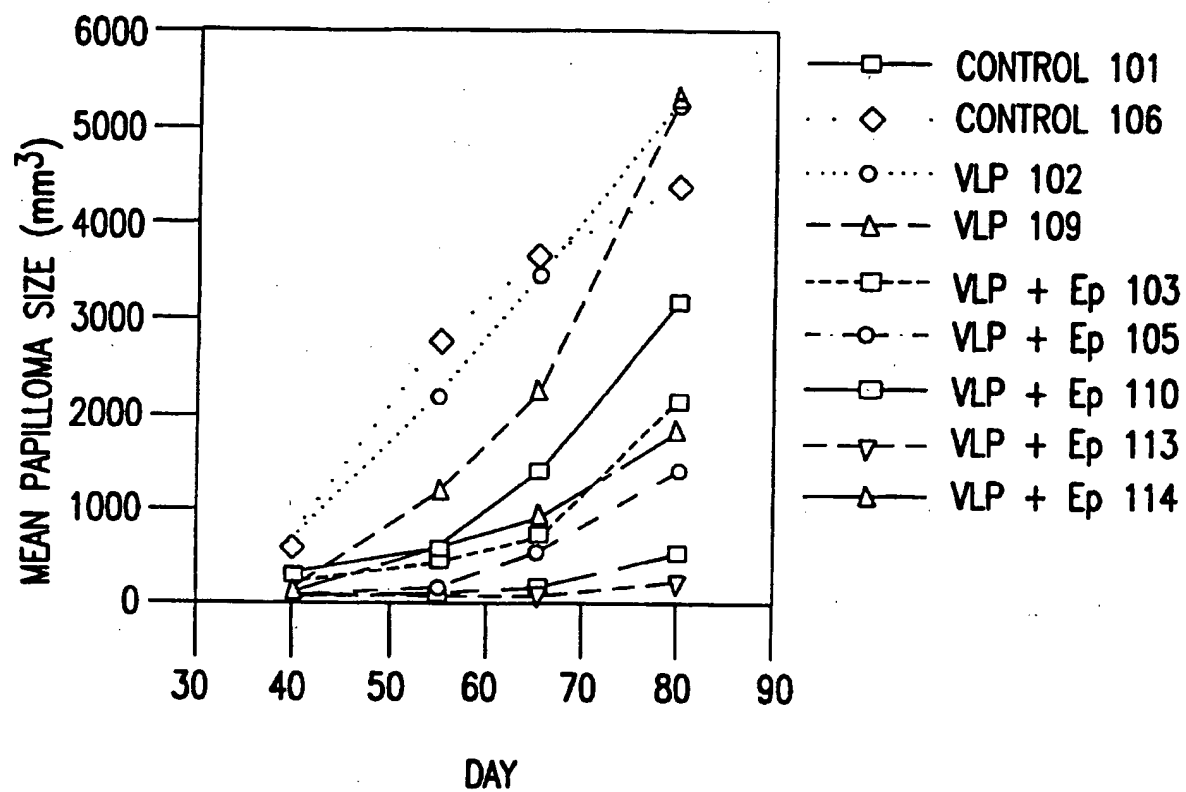


FIG.4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/15820

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/12

US CL : 424/204.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/204.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 5,618,536 A (LOWY et al) 08 April 1997, see entire document.	1-7
X, P	MULLER et al. Chimeric Papillomavirus-like Particles. Virology. 21 July 1997, Vol. 234, pages 93-111, see entire document.	1-7
X --- Y	DE 44 35 907 A1 (GISSMANN et al) 11 April 1996, see entire document.	1-7 --- 8-9
Y	WO 96/26277 A1 (CANTAB PHARMACEUTICALS RESEARCH LIMITED) 29 August 1996, see entire document, especially claims 4 and 13-15.	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

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Authorized officer

MARY E. MOSHER, PH.D.

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INTERNATIONAL ARCH REPORT

Intern application No.
PCT/US97/15820

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BREITBURD et al. Immunization with Viruslike Particles from Cottontail Rabbit Papillomavirus (CRPV) Can Protect Against Experimental CRPV Infection. Journal of Virology. June 1995, Vol. 69, No. 6, pages 3959-3963, see entire document.	1-9

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, DERWENT WORLD PATENTS INDEX. SEARCH TERMS: HPV, HUMAN, PAPILLOMA?, E1, E2, E3, E4, E5, E6, E7, L1, L2, ANTIBODY, ANTIBODIES, ANTIGEN, ANTIGENS, ANTIGENIC, VACCINE, VACCINES, RIBI TRIPLE MIX.